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New Echiniscidae (Heterotardigrada) from Amber Mountain (Northern Madagascar)

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Abstract

A moss sample from the local biodiversity hotspot in lowland rainforest in the vicinity of Amber Mountain, Madagascar, yielded the discovery of two *Echiniscus* C.A.S. Schultze, 1840 species, of which one is new to science. *Echiniscus succineus* **sp. nov.** is related to other members of the *spinulosus* group, but differs from them by the highly complicated structure of the dorsal plates, with intricately thickened parts of the armour forming ornamented pattern. The validity of the intraporal dark rings as a taxonomic trait is discussed in the context of the recovered intraspecific variability for the new taxon. Besides, rare *Echiniscus africanus* Murray, 1907 is reported for the first time from the island.

Key Words

appendages
cuticle
Echiniscus africanus
morphology
pores
spinulosus group
taxonomy

Introduction

Madagascan fauna is widely recognised among biologists for its unprecedented level of endemism and notable species diversity (Myers et al. 2000; Goodman and Benstead 2003, 2005; Holt et al. 2013). However, such enormous biodiversity, like in the majority of the tropical regions of the globe, is in great danger due to massive extirpation of rainforests (Brown and Gurevitch 2004). As an immense fraction of the world's biodiversity remains unexplored (Mora et al. 2011), especially within the taxonomic groups belonging to aquatic meiofauna and limno-terrestrial microfauna, degradation of so unique ecosystems threatens many organisms. Tardigrades are micrometazoans that can be found both in sea and land habitats (Nelson et al. 2015), constituting an important portion of local species abundance. Madagascan tardigrades received almost no attention, with single papers which included two new echiniscid descriptions (Maucci 1993, Pilato and Lisi 2003). As a consequence, only 13 species were recorded from Madagascar. In the present contribution, we present the results of systematic study on two *Echiniscus* C.A.S. Schultze, 1840 species found in the Diana Region located in the Northern Madagascar. *Echiniscus succineus* sp. nov. is described by the means of morphological and genetic analyses and is included within the *spinulosus* group as it exhibits evident pores on all dorsal plates and appendages in the form of spines. *Echiniscus africanus* Murray, 1907, previously reported from South Africa, Angola, Tanzania, and Lesotho (da Cunha and do Nascimento Ribeiro 1964, Binda and Pilato 1995, Middleton 2003, Gąsiorek and Kristensen 2018), is also recorded and illustrated.

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Materials and methods

Sample processing and microscopy

Twenty-two specimens of the new species and a single juvenile of *E. africanus* were extracted from one moss sample, collected from a tree at the edge of lowland rainforest in the vicinity of Amber Mountain (see the subsection *Material examined* for precise location) in December 2018. Dry material was placed and maintained in distilled water for 12 hours, approximately two weeks after collection. Tardigrade extraction procedure followed Stec et al. (2015). Hoyer's medium was chosen for mounting the animals on permanent slides. Fifteen representatives of the new species and the individual of *E. africanus* were examined and photographed under a Nikon Eclipse 50i phase contrast microscope (PCM) associated with a Nikon Digital Sight DS-L2 digital camera. Three specimens of the new species were processed for scanning electron microscopy (SEM) according to the protocol by Stec et al. (2015). All figures were assembled in Corel Photo-Paint X8 software. For deep structures that could not be fully focused in a single photograph, a series of 5–8 images were taken every ca. 0.2 μm and then assembled into a single deep-focus image.

Morphometrics and terminology

All measurements are given in micrometres (μm) and were taken under PCM with Nikon Digital Sight DS-L2 software. Structures were measured only if their orientations were suitable, and structures were not twisted or broken. Body length was measured from the anterior to the posterior end of the body, excluding the hind legs. The *sc* ratio is the ratio of the length of a given structure to the length of the scapular plate (Fontoura and Morais 2011; values italicised in the tables). Morphometric data were handled using the Echiniscoidea ver. 1.2 template available from Tardigrada Register, www.tardigrada.net/register (Michalczyk and Kaczmarek 2013). Detailed measurements are additionally provided as Suppl. material 1. General taxonomy and morphological terminology follow Kristensen (1987), with the further amendments introduced by Gąsiorek et al. (2017, 2019).

Genetic data

DNA was extracted from four individuals of the new species (all animals were examined under 400× magnification in PCM prior to DNA extraction) following a Chelex® 100 resin (Bio-Rad) extraction method by Casquet et al. (2012) with modifications described in detail by Stec et al. (2015). Four molecular markers were sequenced (18S rRNA, 28S rRNA, ITS-2 and *cox1*); see Table 1 for primers and their source details. All fragments were amplified and sequenced according to the protocols described in Stec et al. (2015). Available 18S rRNA and 28S rRNA (dataset identical as in Gąsiorek et al. 2019) + ITS-2 and *cox1 Echiniscus* sequences were uploaded from GenBank to be aligned using default settings of MAFFT version 7 (Katoh et al. 2002; Katoh and Toh 2008). Uncorrected pairwise distances for trimmed alignments (898 bp – 18S rRNA, 670 bp – 28S rRNA, 427 bp – ITS-2, 535 bp – *cox1*) were calculated using MEGA7 (Kumar et al. 2016) and are presented in the Suppl. material 2.

Results

Taxonomic account

Phylum: Tardigrada Doyère, 1840
Class: Heterotardigrada Marcus, 1927
Order: Echiniscoidea Richters, 1926
Family: Echiniscidae Thulin, 1928
Genus: *Echiniscus* C.A.S. Schultze, 1840

Echiniscus africanus Murray, 1907

Fig. 1

Material examined. One juvenile individual. *Terra typica*: South Africa.

Synthetic description. Body yellow and plump, 140 μm long. Cephalic appendages lengths: *cirrus internus* 12.7, cephalic papilla (secondary clava) 5.8, *cirrus externus* 14.7, primary clava 4.1, cirrus *A* 30.3. Trunk appendage formula C-C^d-D-D^d-D^{cd}-E, most spines of similar lengths (16.1–19.0), but spines C^d and D^{cd} much shorter (7.5–9.4), and two additional spicules (2.5–3.1) present at the posterior edge of the scapular plate (29.6). The dorsal plate sculpture of the mixed type (*sensu* Gąsiorek et al. 2019), with large pores surrounded by polygonal edges on the scapular and caudal plates, and endocuticular pillars visible as densely arranged dark dots on the remaining plates, sometimes covered by thick epicuticular ornamentation (Fig. 1).

Table 1. Primers used for sequencing of DNA fragments (one mitochondrial and four nuclear) of *Echiniscus succineus* sp. nov.

DNA fragment	Primer name	Primer direction	Primer sequence (5'-3')	Primer source	PCR programme*
18S rRNA	18S_Tar_1Ff	forward	AGGCGAAACCGCGAATGGCTC	Stec et al. (2017)	Zeller (2010)
	18S_Tar_1Rr	reverse	GCCGCAGGCTCCACTCCTGG	Stec et al. (2017)	
28S rRNA	28S_Eutar_F	forward	ACCCGCTGAACTTAAGCATAT	Gąsiorek et al. (2018)	Mironov et al. (2012)
	28SR0990	reverse	CCTTGGTCCGTGTTTCAAGAC	Mironov et al. (2012)	
ITS-2	ITS3	forward	GCATCGATGAAGAACGCAGC	White et al. (1990)	Wełnicz et al. (2011)
	ITS4	reverse	TCCTCCGCTTATTGATATGC	White et al. (1990)	
cox1	bcdF01	forward	CATTTTCHACTAAYCATAARGATATTGG	Dabert et al. (2008)	Wełnicz et al. (2011)
	bcdR04	reverse	TATAAACYTCDGGATGNCCAAAAAA	Dabert et al. (2008)	

* – All PCR programmes are also provided in Stec et al. (2015).

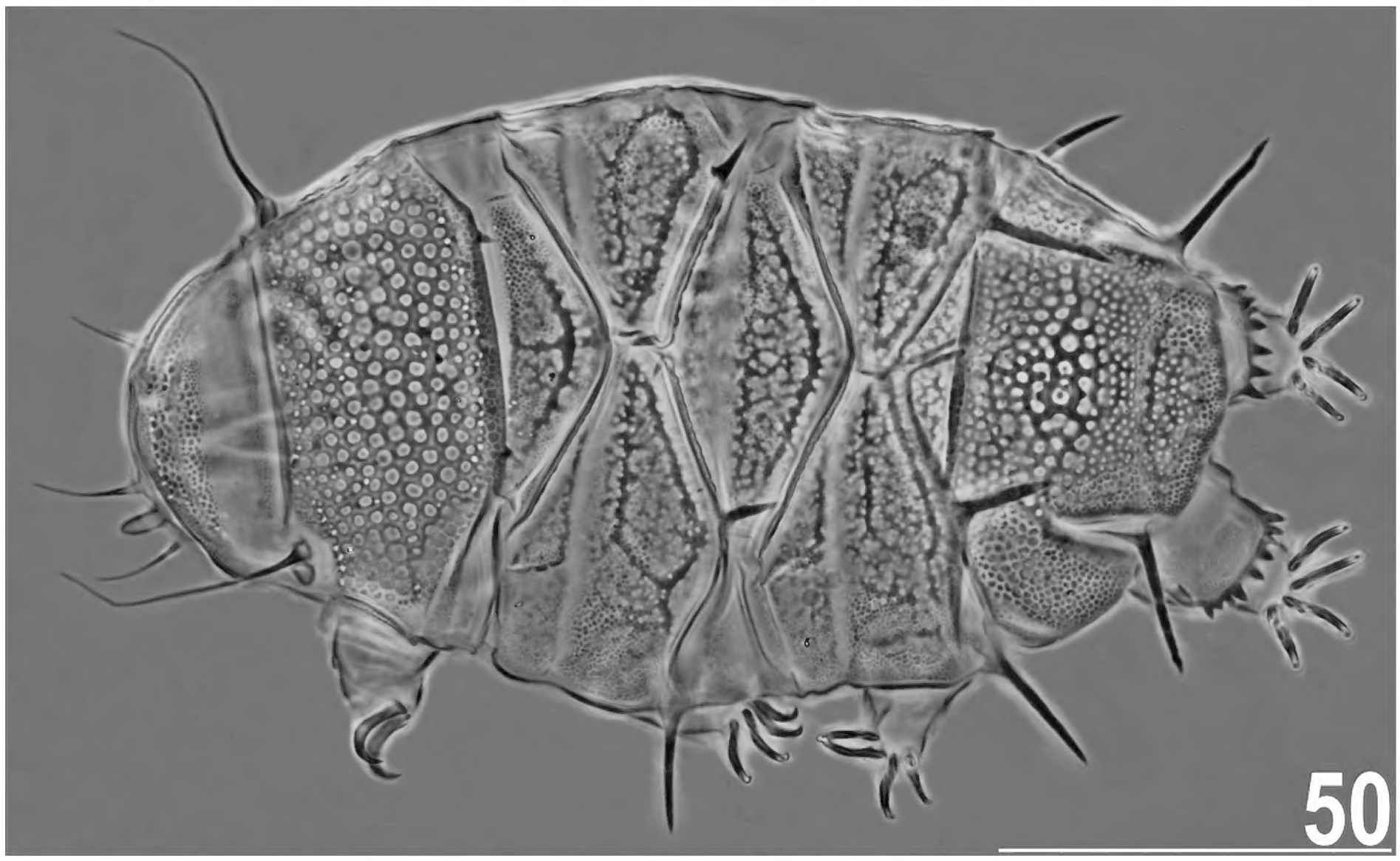

Figure 1. Juvenile of *Echiniscus africanus* Murray, 1907 (PCM). Scale bar: in μm.

Leg appendages and claw lengths: spine on the first leg pair 2.6, papilla on the fourth leg pair 3.9, claws I–IV 7.5–9.3. Serrated fringe on the fourth leg pair consisting of nine teeth.

Distribution. This elusive species has been reported several times only from Southern and Eastern Africa since its description over a century ago (McInnes et al. 2017, Gąsiorek and Kristensen 2018). The record from Vietnam (Węglarska 1962) suggests either disjunctive range or misidentification with *E. semifoveolatus* Ito, 1993, which, however, is not properly delimited from the former species (Qiao et al. 2013).

Remarks. The specimen lacks lateral spines *B* and centrodorsal (mediodorsal) spines C^{cd}, which are characteristic for this species (Murray 1907, 1913). However, both positions are highly instable in terms of the presence/absence of appendages, which was demonstrated for *E. lapponicus* Thulin, 1911 with similar appendage configuration (Dastych 1980).

Echiniscus succineus sp. nov.

http://zoobank.org/D0F1B3CA-D1E6-49C3-8E2F-77973244E9E6

Figs 2–5, Tables 2, 3

Material examined. Holotype (adult female on the slide MG.005.05) and sixteen paratypes (slides MG.005.04–7, including two voucher exoskeletons preserved after DNA extraction on the slides MG.005.28–29 and two specimens on the SEM stub no. 17.11). Except for two paratypes (slide MG.005.04) deposited in the Natural History Museum of Denmark, University of Copenhagen, the entire type series deposited in the Institute of Zoology and Biomedical Research, Jagiellonian University, Poland.

Locus typicus. Lowland rainforest close to the road from Joffreville (Diana Region, Antsiranana Province, Northern Madgascar); coordinates and altitude: 12°30'49"S, 49°10'56"E; 993 m asl. Substratum: moss growing on a tree branch (*ca.* two metres above ground level); collection: December 2018 by W. Witaliński.

Diagnosis. Small representative of the *Echiniscus spinulosus* group with peculiarly complex dorsal plate sculpturing developed as thick epicuticular ridges on scapular, paired segmental and caudal plates. Spines in almost all lateral and dorsal trunk positions. Parthenogenetic.

Description. Adult females and juveniles. Body dark yellow and plump. Red eyes present, dissolved after mounting. External cirri not markedly longer than internal cirri, with swollen cirrophores (Fig. 3C). Primary and secondary clavae (cephalic papillae) of similar lengths. Cirrus *A* short (cirrus *A*/body length ratio below 20%), with short and poorly marked cirrophore. Trunk appendage configuration *(B)-C-*C^{d}*-D-*D^{d}*-E* in adults (Figs 2, 3A–B), reduced to (C^{d})*-*D^{d}*-E* in juveniles. All appendages in form of spines of similar lengths, spines D^{d} and *E* more robust, and sometimes gently serrated or rough (Figs 3A–B). Asymmetry in the development of appendages frequent, one of the spines *B* almost always absent, more rarely one of the spines *C* and *D* absent. Dorsal plates with rather irregularly distributed, large to very large pores (*spinulosus* type; Figs 2, 3). Dark endocuticular rings variously developed: from barely visible on the central portions of median plates (Fig. 3A) to well-developed rings present in pores from different plates (Figs 3B, 4A); they are the

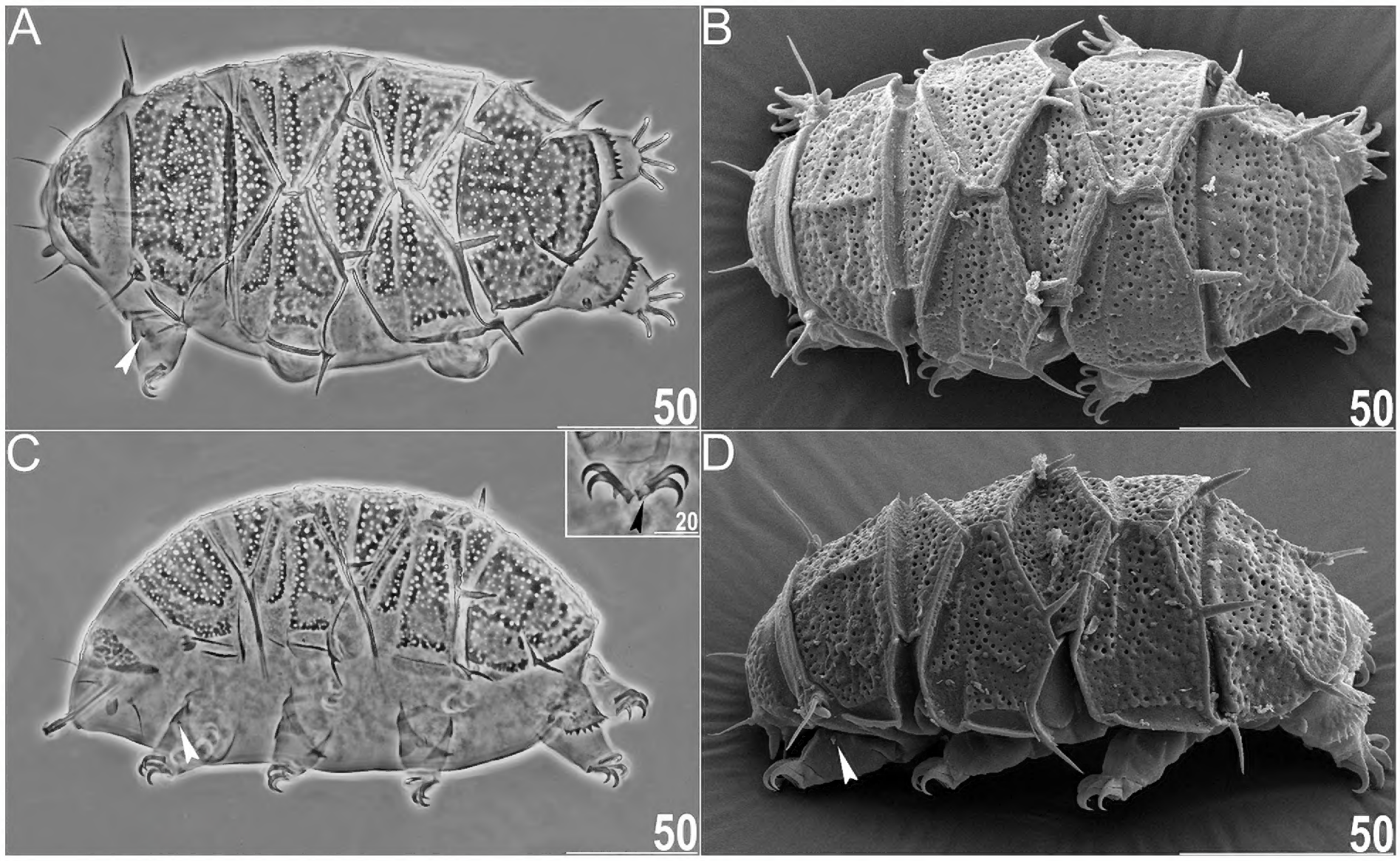

Figure 2. Habitus of adult females of *Echiniscus succineus* sp. nov.: **A** – holotype, dorsal view (PCM); **B** – paratype, dorsal view (SEM); **C** – paratype, lateral view (PCM, insert with the claws of the second leg pair, black arrowhead indicates spur); **D** – paratype, lateral view (SEM). White arrowheads point out spine on the first leg. Scale bars: in μm.

elements of sponge-like endocuticular layer visible under SEM (Fig. 3D, 4B). The level of development of the rings is not associated with life stage, and some individuals do not exhibit intraporal rings. Cephalic plate large, halved, with scarce and minute pores (Figs 2, 3A–C). Cervical (neck) plate present, poreless and developed as grey rectangular belt adjacent to the anterior margin of the scapular plate (Fig. 3A–C). Scapular plate with the system of thick epicuticular extensions, dividing its surface into clearly defined areas of thinner cuticle, being lighter under PCM and slightly concave under SEM (Fig. 3A–C). Median plates I–III large and uniformly dark under PCM, the first and the third plate are unipartite, whereas the second one is bipartite, with its anterior portion being a poorly developed, narrow triangle (Figs 2, 3A–B). At the posterior margins of median plates I–II and paired plates, irregular epicuticular thickenings may be present, especially in larger animals (compare Figs 3A–B, D). Each of paired plates indistinctly divided by a thin smooth band into a narrow anterior portion with condensed epicuticular matrix, and a larger posterior portion with more complex ornamentation pattern. The proximal part of each posterior portion is thick similarly to the anterior portion, but more distal one is thinner, with reduced and less numerous pores (Figs 3A–B, D). Marginally, a single dark epicuticular belt is present, and the second belt appears more centrally (Fig. 3A), however, sometimes it is not discernible (Fig. 3B). Caudal (terminal) plate with typical incisions, rarely sclerotised as if being a prolonged extensions of spine *E* (Fig. 3B). Its epicuticular ornamentation is similar in form to that occurring on the scapular plate (Figs 2, 3A), but may be less developed (Fig. 3B, 3E).

Ventral plates absent, but simple granulation covers the entire venter from the subcephalic to genital zone. Endocuticular pillars minute and not-differentiated in size. Pedal plates and pulvini absent. Spine on the first leg pair minuscule (Figs 2A, 2C–D, 5A), either in the form of usual triangle or blunt (Fig. 2A). Dentate collar on the fourth leg pair present, with short teeth similar in shape (Figs 2, 3B). Papilla on the fourth leg pair present (Figs 2, 3B). External claws spurless, but internal ones bear acute spurs inserted at *ca*. 20–25% of the claw branch and directed downwards (Figs 2C, 5). Claws IV longer than claws I–III.

Larvae and eggs. Unknown.

DNA barcodes. Four genetic markers were represented by single haplotypes. The 18S rRNA sequence (898 bp long, GenBank accession no. MK675903):

GATAACTGTGGTAATTCTAGAGCTAATA-
CATGCAGTAAGCCTTGACCTTTACCGGCAA-
GGCGCAGTTATTAGATCAAAAACCAATCG-
GTTGTGTCTTCGGATGCAGCCGTTAGCTTG-
GTGACTCTGAGTAACCACAGCGAACCG-
TATGGCCTCGTGCTCGACGGTCTGTCAGT-
CAAGCAACTGCCTTATCAGCTTGTTGTTAG-
GTTATATGCCTAACAAGGCTTCAACGGG-

TAACGAACGATCGGGGTCGGATATCGGAGAGG-
GAGCTTGAGAAACGGCTACCACTTCCAAGGAA-
GGCAGCAGGCGCGCAAATTACCCACTCTCGG-
CATGAGGAGGTAGCGATAAAATGTATCGATG-
CGGGGCCATTAGTGCCTTCGTAATCG-
GAATGAGTACACTTTAAATCCTATAACAAG-
GACCTATTGGAGGGCAAGTCTGGTGCCAG-
CAGCCGCGGTAATTCCAGCTCCAATAGCG-
TATATTAATGCTGCTGCGGTTAAAAAGCTCG-
TAGTCGGATCTGGGTTACCGGCGGGTACCG-
CATGTTGCTTCACGCAGCATGTTGTGTAC-
TATACGTGTCGCTTCGGCGGCACTGCCAGTG-
TAATTGTGCCTCACGTAGGTACGTTACGCTG-
GTCGCCGGAACCACGAGCCGGGTTGAGCAG-
CATGCTCTTAATTGAGTGTGTTGTTTACTCG-
GTGCGTTTACTTTGAAAAAATTGGAGTGCT-
CAAAGCAAGCGTACAGTCGCTATGCGGCTT-
GAACAGTGGTGCATGGAATAATGGAATAG-
GGCCTCGGTTCTATTTTGTTGGTTTTAAGA-
TATCGAGGTAATGATTAAGAGGGACAGACGG-
GGACGTTTGTATTGCGACGTTAGAGGTGAAAT-
TCTTGGATCGTCGCAAGACACACTAATGCGAA

The 28S rRNA sequence (767 bp long, GenBank accession no. MK675914):

GCTGGACTTAAGCATATTAATAAGCGGAG-
GAAAAGAAACCAACAGGGATATTCTCAGTAAC-
GGCGAGTGAAGAGAATAcAGCCCAGCGCTGAAT-
CATACTGCTTGCAAGAGTAGTACGACATGTAG-
CGTGAAACTGGCGGCTGTTGATGTTGTCGATG-
CGTGTAAGTCTTCTTGATTGAGGCTCAGTCCCA-
GAGATGGTGCTAGGCCCGTATCGCGCGTGA-
CAAGTACAGCAACGCCCGCTTGTGGAGAGTCAG-
GTTGTTTGGGAACACAATCTAAAGCCGGTGGTA-
CACTCCATCGAAGGCTAAATATGGCCACGAGTC-
CGATAGCGAACAAGTACCGTGAGGGAAAATT-
GAAAAGCACTTTGAAGAGAGAGCGAAATAGT-
GCGTGAAACCGCTTAGAGGCAAGCAGATGGAT-
TCTCGAAGGTGTGCATAGGATTTATTTCCTAGTTCT-
CACGCCACCGCTGTTGTTGACGTGCACCATAC-
GCTGACATTTGGACGCTTGAGATTGGGACTCGT-
GCCTGCTTGAGCTGCTCGGTGTCGGACGTATT-
GAGTTGATTCGTGGCATGCGATAACAGAGCAGAG-
CATTTGTCGTCGCTGTAAAGCGCTGACTGTGGC-
CGCTTGCTGATGCATTGTTGTTGTGGCAAGGCG-
CAAGCTTTGACATGCGATATGTATTGCAACTCG-
GCTATTAGTACCGGCAAGACGACTTCAAGACTC-
GGTGGCGAGTAGACGAACTTCCATCTAAC-
CCGTCTTGAAACACGGACCAAGGGA

The ITS-2 sequence (442 bp long, GenBank accession no. MK675925):

GGTTTTCTGAACGTTAATTCTTCTAACG-
CAAATTGCAGCTGTGATTTTAGTCGCAGC-
TACGCCCGGTTGAGGGTCAGTTGATCATA-
AACTCGCTTGTAACTGTTGTAACTACAAGCG-
CATTGGCTGTTCACATTGACTGCTTCAATGC-
GGCTGATGTGTTAGCTCAAATTGCCAAGCT-
GCCAACAAAGCAGTTTCGGATTTCTTGTTAT-
GTATGCTGCTCTAGCAGGTCGTTGTTTGT-
CAGTACTATGCACTGCTTCAAGATTATTGTG-
CGTGCTGACAAAGCTGCGTATGTGTGCGG-
CAGACAGCATGCGGACCAGTCGTTCGCAT-
GACTCGTCTCTAACGGCATTTGCTTCTCATA-
CACATATAACAAACCAATCATTTTTGTGACCT-
CAACTCGGACGAGACTACCCGCTGAATTTA-
AGCATATCAATAAGCGGAGGAA

The *cox1* sequence (614 bp long, GenBank accession no. MK649675):

TACTTTATATTTTTATTTTTTGGTTTATGGGCT-
GCTTCTGTTGGTTCAAGTTTAAGGTTTTTAAT-
TCGAACTGAATTATCTCAACCAGGAATTTGGT-
TAGGCGACGAGCATTTATATAATGTCTTAGT-
TACTTCCCATGCTTTAATTATAATTTTTTTTATGG-
TAATACCAATCTTAATTGGTGGTTTTGGTAATT-
GATTAATTCCTATTATAATTGGTGCCCCGGATAT-
GTCATTTCCTCGAATAAATAATTTAAGTTTTTG-
GCTTTTACTACCTTCTTTGCTTTTGCTATT-
GATTTCTTCTAATATTAGATCTGGTGTGG-
GCTCTGGTTGAACTTTATACCCACCTTTATCT-
GAATTTATTGGTCATTCTAATTATACTGTTGA-
TATGGCTATTTTTTCTTTCCATGTTGCTGGT-
GCTTCTTCTATTTTAGGTGCTATTAATTTTATT-
ACTACTATTTTGAATATACGTTTTTTTTCTTTA-
AATATAGAACAGTTATCTTTATTTGTTT-
GATCTGTTTTGATTACTGCTATCTTACTA-
ATTTTATCTTTACCTGTTTTAGCCGGCGG-
TATTACTATATTATTGTTAGATCGTAATTTT-
AATAGTTCTTTTTT

Etymology. From Latin *succineus* = amber, referring to the *locus typicus* near Amber Mountain. An adjective in the nominative singular.

Comparative discussion: This is the second known member of the *spinulosus* group with scapular, paired segmental and caudal (terminal) plates markedly ornamented. Similar system of epicuticular thickenings exists in *E. ornamentatus* Gąsiorek & Kristensen, 2018 described recently from Tanzania, but an adult specimen of *E. succineus* sp. nov. is easily distinguishable from the latter taxon based on: the appendage configuration (*A-(B)-C-C^d-D-D^d-E* in *E. succineus* sp. nov. *vs A-(B)-C-D-D^d-E* in *E. ornamentatus*), the location of epicuticular ornamentation on the dorsal armour (except for the median plates, all trunk plates ornamented in *E. succineus* sp. nov. *vs* only scapular and caudal plates ornamented in *E. ornamentatus*), and the pore morphology (very large pores, sometimes with endocuticular dark rings in *E. succineus* sp. nov. *vs* minute pores, always without endocuticular dark rings in *E. ornamentatus*). The claws II–IV and all claw spurs seem to be relatively longer in *E. succineus* sp. nov. with respect to *E. ornamentatus* (compare values from Table 2 with table 4 from Gąsiorek and Kristensen (2018)), but given the low number of collated individuals, these traits are not included in the differential comparison.

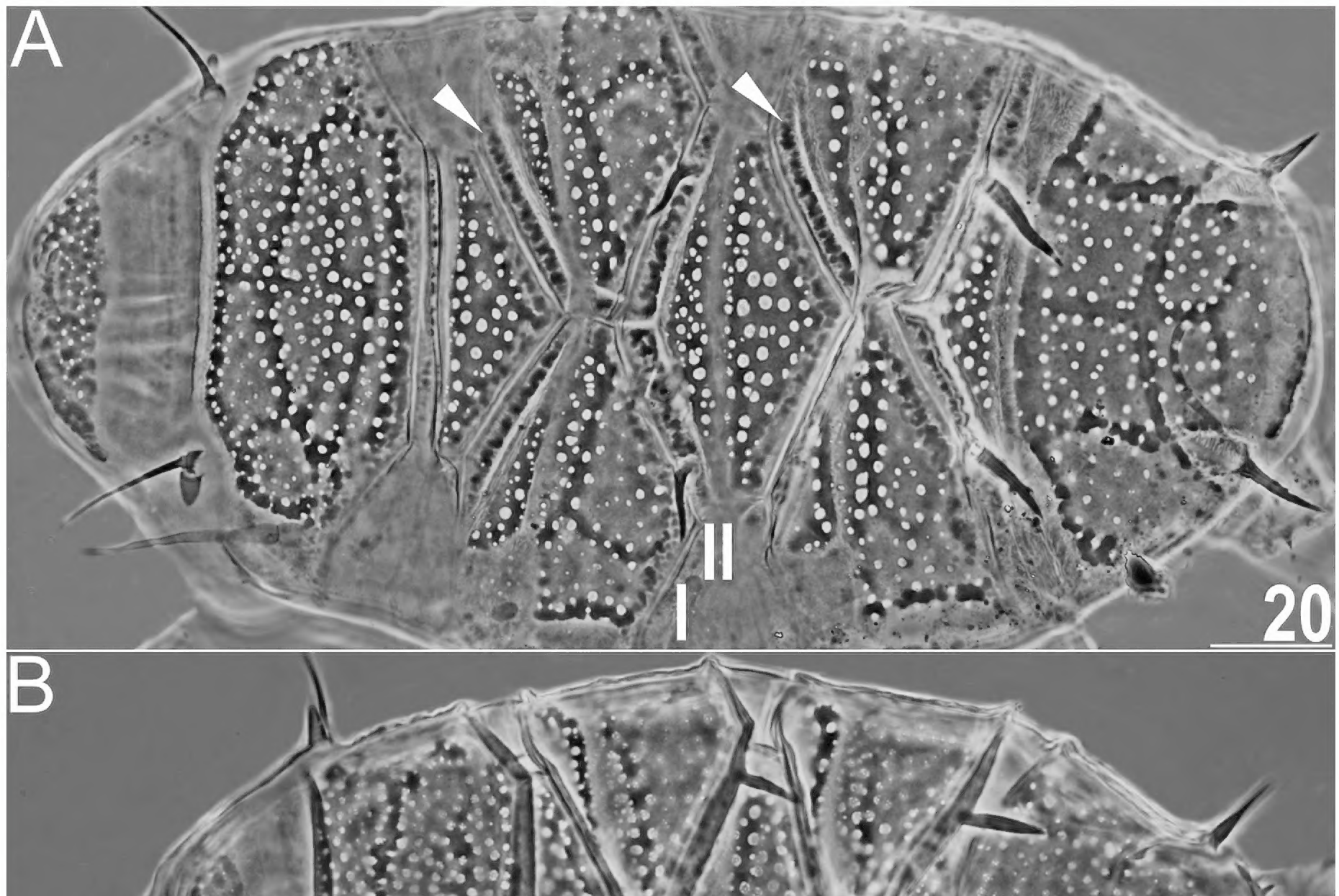

Figure 3. Detailed sculpturing of the dorsal plates of *Echiniscus succineus* sp. nov.: **A** – paratype, dorsal view (PCM, arrowheads indicate epicuticular thickenings); **B** – paratype, smaller specimen, dorsal view (PCM); **C** – scapular plate (SEM); **D** – portion of the second paired segmental plate (SEM); **E** – caudal plate (SEM). Roman numerals signify lateral ornamented belts. Scale bars: in μm.

Three other species are similar to *E. succineus* sp. nov. in overall morphology: *E. marginatus* Binda & Pilato, 1994, *E. scabrospinosus* Fontoura, 1982 and *E. tropicalis* Binda & Pilato, 1995. *E. succineus* sp. nov. differs from:

- *E. marginatus*, reported from Hawaii Archipelago, by the appendage configuration (*A-(B)-C-C^d-D-D^d-E* in *E. succineus* sp. nov. *vs A-(C)-(D)-D^d-E* in *E. marginatus*), and the morphology of posterior portions of me-

Table 2. Measurements [in µm] of selected morphological structures of adult females (the 3rd and older instars) of *Echiniscus succineus* sp. nov. mounted in Hoyer's medium. N – number of specimens/structures measured, Range refers to the smallest and the largest structure among all measured specimens; SD – standard deviation.

Character	N	Range						Mean		SD		Holotype	
		µm			*sc*			µm	*sc*	µm	*sc*	µm	*sc*
Body length	12	156	–	221	457	–	586	196	535	18	39	204	533
Scapular plate length	12	32.1	–	38.9		–		36.6	–	2.1	–	38.3	–
Head appendages lengths													
Cirrus *internus*	12	9.0	–	16.2	26.4	–	45.5	12.9	35.2	1.9	4.6	13.8	36.0
Cephalic papilla	12	5.8	–	8.1	17.3	–	22.8	7.0	19.1	0.6	1.6	7.7	20.1
Cirrus *externus*	12	12.4	–	18.7	34.6	–	52.5	15.2	41.5	1.9	4.7	17.7	46.2
Clava	12	4.5	–	7.6	13.2	–	21.3	6.1	16.7	0.8	2.0	6.6	17.2
Cirrus *A*	12	17.8	–	32.7	48.6	–	88.2	23.6	64.5	4.6	12.3	21.4	55.9
Cirrus *A*/Body length ratio	12	8%	–	18%		–		12%	–	3%	–	10%	–
Body appendages lengths													
Spine *B*	8	6.9	–	11.6	18.1	–	32.3	10.3	27.6	1.6	4.7	10.7	27.9
Spine *C*	12	8.7	–	16.2	25.5	–	42.4	13.3	36.1	2.3	5.1	16.1	42.0
Spine C^d	12	5.3	–	15.7	15.5	–	44.1	12.0	32.6	2.8	6.9	13.8	36.0
Spine *D*	11	10.6	–	14.9	28.1	–	40.8	12.8	34.5	1.6	4.5	14.2	37.1
Spine D^d	12	14.3	–	21.7	37.4	–	63.6	16.9	46.5	2.0	7.1	16.4	42.8
Spine *E*	12	11.4	–	16.8	29.3	–	48.9	14.1	38.6	1.7	5.8	14.3	37.3
Spine on leg I length	12	1.7	–	2.9	4.8	–	7.7	2.2	6.1	0.3	0.9	2.1	5.5
Papilla on leg IV length	12	3.2	–	4.8	9.2	–	13.5	3.9	10.7	0.5	1.2	4.1	10.7
Number of teeth on the collar	11	8	–	12		–		9.8	–	1.1	–	10	–
Claw 1 lengths													
Branch	12	8.5	–	10.7	22.1	–	28.2	9.6	26.1	0.7	1.7	10.7	27.9
Spur	5	1.5	–	2.3	4.2	–	6.7	1.8	5.2	0.3	1.0	2.0	5.2
Spur/branch length ratio	5	16%	–	24%		–		19%	–	3%	–	19%	–
Claw 2 lengths													
Branch	12	8.0	–	10.1	23.7	–	27.6	9.3	25.5	0.6	1.3	10.1	26.4
Spur	6	1.5	–	2.3	4.2	–	5.9	1.8	5.0	0.3	0.7	1.9	5.0
Spur/branch length ratio	6	16%	–	23%		–		19%	–	2%	–	19%	–
Claw 3 lengths													
Branch	12	8.1	–	10.5	23.3	–	27.4	9.3	25.4	0.7	1.4	10.5	27.4
Spur	8	1.5	–	2.0	4.1	–	5.2	1.7	4.7	0.2	0.4	2.0	5.2
Spur/branch length ratio	8	16%	–	21%		–		18%	–	2%	–	19%	–
Claw 4 lengths													
Branch	11	10.1	–	12.9	27.5	–	33.7	11.4	31.0	0.9	1.8	12.9	33.7
Spur	4	1.8	–	2.4	5.1	–	7.0	2.1	5.9	0.3	0.9	?	?
Spur/branch length ratio	4	17%	–	22%		–		19%	–	2%	–	?	–

dian plates I–II (narrow and with irregular thickenings in *E. succineus* sp. nov. *vs* broad, solid and poreless in *E. marginatus*, see Pilato et al. 2008);

- *E. scabrospinosus*, known from Western Palaearctic and Afrotropical realm, by the appendage configuration (*A-(B)-C-C^d-D-D^d-E* in *E. succineus* sp. nov. *vs A-(C)-(D)-D^d-E* in *E. scabrospinosus*), and the morphology of posterior portions of median plates I–II (with irregular thickenings in *E. succineus* sp. nov. *vs* porous in *E. scabrospinosus*, see Pilato et al. 2008);
- *E. tropicalis*, recorded from the Seychelles, by the appendage morphology (spines in *E. succineus* sp. nov. *vs* very short, triangular spicules in *E. tropicalis*), and spurs on the internal claws IV (identical to spurs on internal claws I–III in *E. succineus* sp. nov. *vs* larger and better developed spurs IV, more divergent from the claw branches than on internal claws I–III in *E. tropicalis*).

Comparative genetic analysis: The uncorrected pairwise distances between *E. succineus* sp. nov. and the remaining *Echiniscus* spp. were as follows: (1) 18S rRNA – from 0.5% (*E. manuelae* da Cunha & do Nascimento Ribeiro, 1962) to 2.5% (*E. testudo* (Doyère, 1840)); (2) 28S rRNA – from 2.7% (*E. manuelae*) to 6.1% (*E. testudo*); (3) ITS-2 – from 17.6% (*E. testudo*) to 22.9% (*E. blumi* Richters, 1903); (4) *cox1* – from 15.7% (*E. merokensis* Richters, 1904) to 18.5% (*E. granulatus* (Doyère, 1840)).

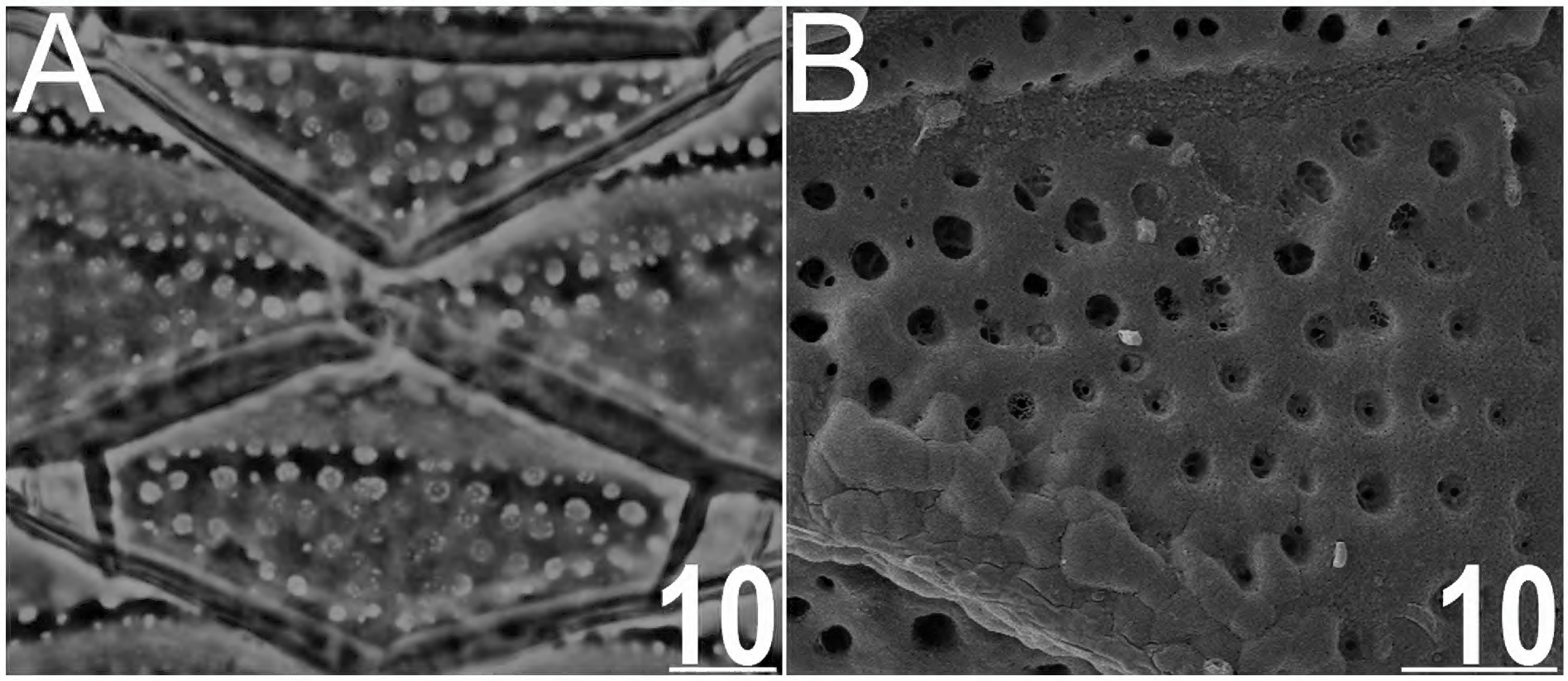

Figure 4. Endocuticular (intraporal) rings of *Echiniscus succineus* sp. nov.: **A**– median plates I and II (PCM); **B** – central portion of the second paired segmental plate (SEM). Scale bars: in µm.

Table 3. Measurements [in µm] of selected morphological structures of juveniles (the 2nd instar) of *Echiniscus succineus* sp. nov. mounted in Hoyer's medium. N – number of specimens/structures measured, Range refers to the smallest and the largest structure among all measured specimens; SD – standard deviation.

Character	N	Range						Mean		SD	
		µm			*sc*			µm	*sc*	µm	*sc*
Body length	3	128	–	160	508	–	533	141	518	17	13
Scapular plate length	3	25.2	–	30.0		–		27.1	–	2.6	–
Head appendages lengths											
Cirrus *internus*	3	6.0	–	12.6	23.8	–	42.0	8.6	31.1	3.5	9.6
Cephalic papilla	3	4.0	–	5.6	15.9	–	18.7	4.7	17.1	0.8	1.4
Cirrus *externus*	3	8.5	–	14.7	33.7	–	49.0	10.9	39.7	3.3	8.2
Clava	3	3.8	–	5.7	14.6	–	19.0	4.5	16.3	1.1	2.3
Cirrus *A*	3	13.9	–	23.5	55.2	–	78.3	17.4	63.5	5.3	12.9
Cirrus *A*/Body length ratio	3	11%	–	15%		–		12%	–	2%	–
Body appendages lengths											
Spine C^d	1	9.8	–	9.8	32.7	–	32.7	9.8	32.7	?	?
Spine D^d	3	11.6	–	18.0	46.0	–	60.0	14.4	52.8	3.3	7.0
Spine *E*	3	6.7	–	13.1	26.6	–	48.7	10.8	39.6	3.6	11.6
Spine on leg I length	2	1.2	–	2.4	4.8	–	8.0	1.8	6.4	0.8	2.3
Papilla on leg IV length	3	1.9	–	3.3	7.5	–	11.0	2.7	9.8	0.7	1.9
Number of teeth on the collar	3	7	–	8		–		7.7	–	0.6	–
Claw 1 lengths											
Branch	3	6.0	–	8.0	23.8	–	26.7	6.8	25.1	1.0	1.4
Spur	0		?			?		?	?	?	?
Spur/branch length ratio	0		?			–		?	–	?	–
Claw 2 lengths											
Branch	3	5.6	–	8.0	22.2	–	26.8	6.9	25.2	1.2	2.6
Spur	2	0.6	–	0.7	2.4	–	2.7	0.7	2.5	0.1	0.2
Spur/branch length ratio	2	10%	–	11%		–		10%	–	1%	–
Claw 3 lengths											
Branch	3	5.8	–	8.4	23.0	–	28.0	6.8	25.1	1.4	2.6
Spur	2	0.9	–	1.3	3.6	–	4.3	1.1	4.0	0.3	0.5
Spur/branch length ratio	2	15%	–	16%		–		15%	–	0%	–
Claw 4 lengths											
Branch	3	6.9	–	9.7	26.4	–	32.3	7.8	28.7	1.6	3.2
Spur	0		?			?		?	?	?	?
Spur/branch length ratio	0		?			–		?	–	?	–

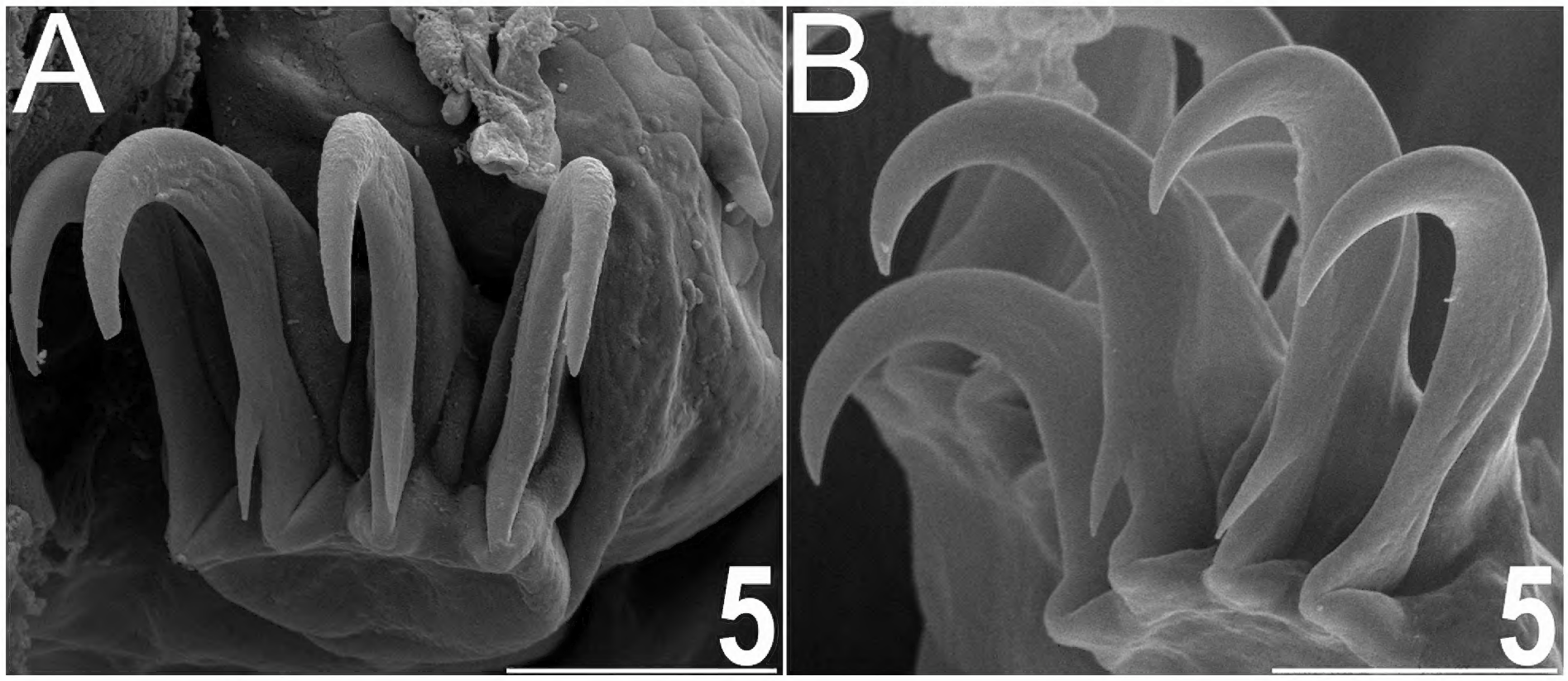

Figure 5. Claws of *Echiniscus succineus* sp. nov. (SEM): **A** – first leg pair (small spine I visible in the upper right corner); **B** – fourth leg pair. Scale bars: in μm.

Discussion

The knowledge on the Madagascan tardigrade fauna is limited. Most of the taxa recorded by Maucci (1993) are now recognised as species complexes, thus the presence of type species being typical Palearctic elements on Madagascar is highly dubious (*e.g.* Guidetti et al. 2019, Morek et al. 2019). In result of the paucity of studies, its microfauna is of unknown origin at present (Yoder and Nowak 2006). *E. africanus* inhabits unchanged lowland rainforest on the island, which suggests some influence of Afrotropical fauna on Madagascan biota. Interestingly, species most similar in terms of morphology to *E. succineus* sp. nov. also occur in the tropical and subtropical zone.

Traditional species delineation in many Echiniscidae relied on the appendage configuration, however the *spinulosus* group poses a significant problem in this context as characterised by high variability in symmetry and presence of trunk spines. Pilato et al. (2008) introduced the presence of intraporal rings as a specific trait and an additional criterion in the taxonomy of the *spinulosus* group. Nevertheless, the variability in the development of these structures, shown for the first time to be endocuticular elements of the sponge-like layer, in *E. succineus* sp. nov., suggests the need for re-assessment of the validity of this feature. Considering the fact of explicitly emphasised significance of the dorsal plate sculpturing for classification and understanding the phylogeny of the *Echiniscus* lineage (Gąsiorek et al. 2019), the clarification of this issue would be desirable. There is a possibility that some species within the *spinulosus* complex always exhibit or do not exhibit dark rings, whereas other taxa are more inconstant in that respect.

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Supplementary material 1

Detailed tables with morphometry

Authors: Piotr Gąsiorek, Katarzyna Vončina

Data type: morphometric data

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Link: https://doi.org/10.3897/evolsyst.3.33580.suppl1

Supplementary material 2

Uncorrected pairwise distances

Authors: Piotr Gąsiorek, Katarzyna Vončina

Data type: genetic data

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